APPLICATIONS

Investigation of Buffer pH Change During Storage in Solvent Reservoirs with Different Closures and Its Potential Mitigation by the Use of SecurityCAP™

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Zeshan loves to collect watches and the Back to the Future Trilogy. He has twin boys which drive him crazy! He is an Apple® fanboy for life and he likes being in the lab more than anywhere else.

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Abstract
Losses of volatile buffer components to the atmosphere can result in poor laboratory air quality, as well as, the potential to cause health issues for laboratory staff. There are also implications in the potential variability in chromatography as mobile phase pH changes.

Introduction
The control of mobile phase pH is an important consideration in reversed phase HPLC solvent systems. A drift in mobile phase pH can result in retention time instability which may then cause peaks to be misidentified. Peaks may also appear to be distorted, and in extreme cases peaks may co-elute. When utilizing mass spectrometry as a detector, it is essential that all mobile phase components, including those used for pH adjustment or buffering, to be volatile. The use of volatile buffer components does however mean that there is the potential for losses of these components during storage and use, which can then result in pH drift.

There are a variety of commonly used approaches to capping solvent reservoirs used with HPLC systems, some of which allow the reservoir to be open to the air. This has the potential to cause issues as dust or other airborne particles can contaminate the mobile phase, risking blockage of system filters or even the HPLC column itself. There are also potential exposure risks for users. Solvent reservoirs are often placed on top of HPLC systems to allow gravity to assist in providing flow to the pump head. Such positioning together with commonly used caps increase the risk of splashing or spillage of solvents.

In this tech note we investigate the use of a variety of different solvent bottle closures on mobile phase pH for an ammonium bicarbonate system. We also look at the potential impact on chromatography for a formic acid system.

Experimental Conditions
In the first study, a 5mM ammonium bicarbonate buffer at pH 11 was prepared 5 times. Each 1 liter solution was stored in a bottle with a different closure, ranging from a completely closed cap, open bottle, improvised aluminium foil closure, and SecurityCAP. The pH of the solutions was recorded and then checked after 7 days.

In the second study, where 0.1 % formic acid was prepared, one portion used fresh, the others were stored in bottles with closures as detailed in the first study, for 7 days. The mobile phase was then used along with 0.1 % formic acid in acetonitrile, which was prepared fresh on day 1, and again at day 7.

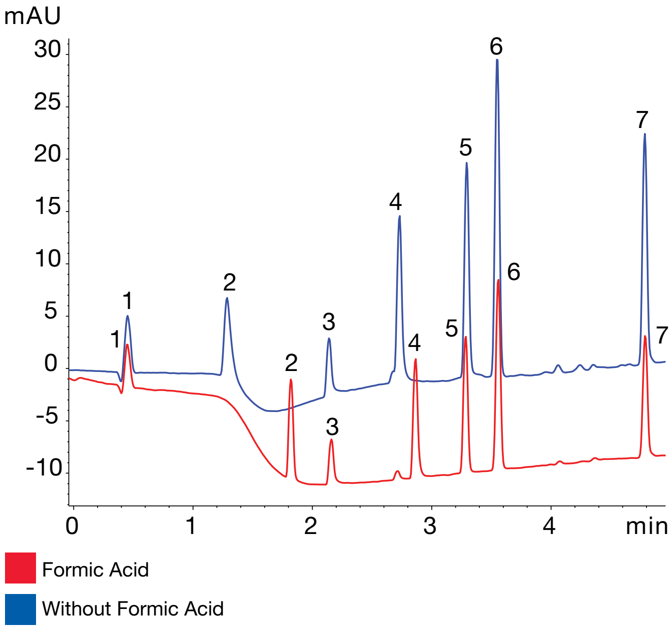
HPLC Conditions

Column: Kinetex® 2.6 µm EV0-C18
Dimensions: 50 x 4.6 mm
Part No.: 00B-4752-OE
SecurityCAP: Mobile Phase Safety filter and cap
Part No.: AC2-1245
Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Acetonitrile (always fresh)
Gradient: 5 % to 95 % over 5 minutes @ 1.25 mL/min
Flow Rate: 1.25 mL/min
Temperature: 40 °C
Analytes:
1. Uracil
2. Pindolol
3. Chlorpheniramine
4. Nortriptyline
5. 3-Methyl, 4-Nitrobenzoic Acid
6. 2-Hydroxy, 5-Methylbenzaldehyde
7. Hexanophenone

Table 1.

Bottle	Initial Solvent Weight (g)	Solvent Weight After 10 Days (g)	% Difference
SecurityCAP	11.01	10.82	1.7
Fully Closed Cap	11.17	10.99	1.6
3-Port Cap	11.13	10.78	3.1
No Cap	11.39	10.01	12.1
Foil	11.07	10.51	5.1

Only peaks 2 and 4 were monitored as they are the analytes affected by the loss of formic acid as seen in the chromatogram below.



Experimental Conditions *(cont'd)*

Table 2.

Bottle	Peak 2 RT	Peak 4 RT	% difference Peak 2	% difference Peak 4
Initial	1.821	2.864	NA	NA
SecurityCAP	1.809	2.853	0.66	0.38
Foil	1.807	2.852	0.77	0.42
3-Port Cap	1.804	2.845	0.93	0.66
Fully Closed Cap	1.781	2.69	2.20	6.08
No Cap	1.792	2.835	1.59	1.01

Results and Discussion

Looking at the data for the ammonium bicarbonate pH change (**Table 1**) SecurityCAP™, with its sealed connection to the HPLC connecting tubing and attached air filter demonstrated a 0.1 % change relative to the control (a closed cap), the absolute change seen was 1.7 %. This compares very favourably with a traditional 3 port cap, where the tubing is not sealed to the cap, which showed a change of 3.1 %. An improvised aluminium closure showed a larger change, at 5.1 % whilst the uncapped bottle showed the largest change at 12.1 %. This highlights the importance of both the design of the ports which hold the connecting tubing, and the air filter port, in reducing the loss of volatile components from the mobile phase reservoir.

In the second study, performance of a formic acid mobile phase system based on the cap used for storage was compared. The first experiment analyzed the retention time of analytes with and without formic acid. Changes in the retention of pindolol (peak 2) and nortriptyline (peak 4) were observed. The data for these 2 compounds was then compared in **Table 2.** A similar trend is seen with the ammonium bicarbonate system, mobile phase stored in the reservoir capped with SecurityCAP exhibited the smallest change, in this study the foil closure performed better than the traditional 3-port cap, with the uncapped bottle again showing the largest changes. One additional point to note is that the data for the completely closed cap is not consistent with the rest of the data set. This data was confirmed by replicating the chromatograms, these replicates were found to be consistent. The different results seen here were not explored further, and cannot be explained at this time.

As well as showing smaller losses of volatile buffer components, an added benefit of the SecurityCAP is that should the solvent reservoir be accidentally tipped during installation or during transportation the cap is completely leak free, preventing accidental spillage or leakage. Also, the tubing which delivers solvent to the HPLC pump is securely held in place. This added protections protect it from moving above the solvent level, which would allow air to be drawn into the system.

Conclusion

The SecurityCAP featured in this technical note provides the best option of those tested for reducing the loss of the two volatile mobile phase additives, ammonium bicarbonate and formic acid. It also prevents the ingress of airborne particulates due to the air filter incorporated in the design. The use of such caps reduces employee exposure to volatile mobile phase additives, both airborne and through accidental spillage.

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...breaking with tradition℠

SecurityCAP™ Ordering Information

Starter Kits

SecurityCAP Mobile Phase (Eluent) Safety Starter Kits

Part No.	Description
AC2-1245	2-port GL45 Cap and 6-month Safety Filter
AC2-4245	2-port GL45 Caps (x4) and 6-month Safety Filters (x4)
AC2-4240	2-port Merck S40 Caps (x4) and 6-month Safety Filters (x4)
AC2-1345	3-port GL45 Cap and 6-month Safety Filter
AC2-4345	3-port GL45 Caps (x4) and 6-month Safety Filters (x4)
AC2-4445	4-port GL45 Cap (x1) and 2-port Caps (3x) and 6-month Safety Filters (x4)
AC2-1445	4-port GL45 Cap and 6-month Safety Filter
AC2-1545	5-port GL45 Cap and 6-month Safety Filter
AC2-1561	5-port S60/S61 Cap and 6-month Safety Filter

SecurityCAP Waste Safety Starter Kits

Part No.	Description	Unit
AC1-1245	2-port GL/DIN45 Cap and 6-month Exhaust Filter and Barbed Connector	ea
AC1-1545	5-port GL/DIN45 Cap and 6-month Exhaust Filter	ea
AC1-1551	5-port DIN51 Cap and 6-month Exhaust Filter	ea
AC1-1561	5-port S61 Cap and 6-month Exhaust Filter	ea

Replacement Filters

SecurityCAP Mobile Phase Safety Filters

Part No.	Description	Unit
AC2-0161	6-month Capacity, ¼ in.-28 Threads	ea
AC2-0961	6-month Capacity, ¼ in.-28 Threads	10/pk

SecurityCAP Waste Safety Filters

Part No.	Description	Unit
AC1-0161	6-month Exhaust Filter for SecurityCAP, ¼ in.-28 Threads	ea
AC1-0361	6-month Exhaust Filter for SecurityCAP, ¼ in.-28 Threads	3/pk
AC1-0162	6-month Exhaust Filter for Wide-port Caps, GL14 Threads	ea
AC1-0362	6-month Exhaust Filter for Wide-port Caps, GL14 Threads	3/pk

Fittings and Accessories

SecurityCAP Fittings

Part No.	Description	Unit
AC3-1101	for 1/16 in. or 2.0 mm ID Tubing, ¼ in.-28 Threads (POM), blue	ea
AC3-1201	for 2.3-2.6 mm ID Tubing, ¼ in.-28 Threads (POM), white	ea
AC3-2101	for ⅛ in. ID Tubing, ¼ in.-28 Threads (POM), black	ea

SecurityCAP Connectors

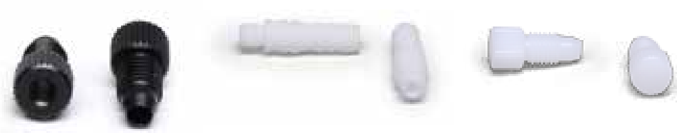
Part No.	Description	Unit
AC3-1001	Barbed Connector, for 5-8 mm ID Tubing (PTFE), white	ea
AC3-1301	Y-connector, for 6-8 mm ID Tubing (POM), white	ea

SecurityCAP Adapter

Part No.	Description	Unit
AC2-1138	Cap Thread Adapter, PTFE, GPI/GL 38 Female to GL45 Male	ea

SecurityCAP Sealing Plug

Part No.	Description	Unit
AC3-2001	¼ in.-28 Threads (POM), white	ea



POM = polyoxymethylene
PTFE = polytetrafluoroethylene (Teflon®)

SecurityCAP Waste Safety Compatibility Table

	Phenomenex SecurityCAP Filters	
Supplier	**ea**	**3/pk**
SCAT® Safety Waste Caps	AC1-0162	AC1-0362
AIT® SmartCaps™	AC1-0162	AC1-0362
Agilent® InfinityLab Stay Safe Caps	AC1-0162	AC1-0362
VICI® Waste Caps	AC1-0161	AC1-0361
Canary-Safe™ Safety Caps	AC1-0162	AC1-0362
DURAN® DG Safety Caps	AC1-0162	AC1-0362

guarantee

If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

APPLICATIONS

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Kinetex EVO is patented by Phenomenex. U.S. Patent Nos. 7,563,367 and 8,658,038 and foreign counterparts.

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